

# Herbicide-Tolerant Crops—Real Farmer Opportunity or Potential Environmental Problem?<sup>‡</sup>

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**Abstract:** Classical breeding, *in-vitro* selection and genetic engineering techniques have produced herbicide-tolerant crops. Commercial adoption of these cultivars in North America provides tolerance predominantly to non-selective herbicides for novel weed-control strategies. Of special value is the integration of these crops in minimum tillage situations, maintaining of a wide range of herbicides with different modes of action to provide a variety of opportunities for weed-control management. Associated with these special crops are a series of environmental issues which at present limit the rate of commercial development in Europe. Unlike the successful performance of herbicide tolerance in the crops, these strategic issues are much more difficult to resolve for technical, political, ethical and moral reasons. The primary concerns are the feasibility of controlling the volunteer crop and the opportunity for indiscriminate introgression of the herbicide-tolerance gene into agricultural and natural ecosystems. It is unlikely that these questions will be resolved without scaling-up field experiments to include the detection of herbicide-tolerance genes. The economic and management implications of herbicide-tolerant crops require special consideration in view of the necessity to integrate conventional and transgenic crops in new cropping systems. © 1998 SCI

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## 1 INTRODUCTION

The genetic improvement of crops has not traditionally given special attention to the nature of the herbicide response. Instead, herbicides have been discovered, screened and formulated to provide selectivity and efficacy of weed control in a range of crops. During the last 20 years, techniques in plant biotechnology have per-

mitted the characterisation, isolation and selection of valuable genes for plant improvement. The herbicide-tolerance trait has featured strongly in such research for several reasons. Frequently the physiological, biochemical and genetical bases of herbicide tolerance are understood and this trait provides a phenotypic marker which is easily assessed. In addition, allied to the strategic research are commercial ambitions to develop new herbicide-tolerant cultivars that will permit a greater use of existing environmentally benign herbicides against the backdrop of increasing costs associated with new herbicide discovery.

An examination of the literature surrounding herbicide-tolerant crops illustrates the focus and direction of research. The early dominant theme covered the

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state of the art with regard to the mechanisms associated with the production of transgenic and non-transgenic herbicide-tolerant crops.<sup>1–7</sup> Secondary themes, in terms of the number of publications, considered the environmental impact of herbicide-tolerant crops.<sup>8–13</sup> With the commercialisation of herbicide-tolerant crops in North America and the resultant food commodities available in the world market place, new issues are raised which directly affect European agriculture. The object of this review is to consider the opportunities which herbicide-tolerant crops might present to farmers and to examine the environmental issues which surround the integration of herbicide-tolerant crops in arable agriculture.

## 2 OPPORTUNITIES

### 2.1 North America

#### 2.1.1 Background

Beyond small-scale field trials, one of the earliest and best-publicised examples of herbicide-tolerant crop cultivars was the Canadian-grown triazine-tolerant rapeseed grown between 1982 and 1990.<sup>14</sup> The triazine-tolerant trait was obtained from a weedy biotype of *Brassica campestris* L. and transferred via classical plant-breeding techniques to Polish rape, *B. campestris* and Argentina rapeseed (*B. napus* L.).<sup>15</sup> While triazine-tolerant cultivars did not prove to be a great commercial success, (c.3.2% of total rapeseed area in 1987) they did enable farmers to grow rapeseed where triazine soil residues remained and allowed the control of competitive Brassica weeds such as *Sinapis arvensis* L. (wild mustard) and *Thlapsi arvense* L. (field pennycress) in addition to grass weeds, e.g. *Setaria viridis* (L.) Beauv., (yellow foxtail). Unfortunately, the triazine-tolerance trait had a physiological cost which was manifest via lower seed yields and oil quality.<sup>16</sup> However, triazine-tolerant rapeseed exposed Canadian farmers to the specialist niche market of herbicide-tolerant cultivars which currently prevails. In addition, the vulnerability of herbicide-tolerant cultivars to swift obsolescence was demonstrated with the arrival of the selective herbicide ethametsulfuron for *S. arvensis* control. Grass weeds were controlled using a range of graminicides including sethoxydim and fluazifop.

#### 2.1.2 Current examples of herbicide-tolerant crops

In the United States, the first herbicide-tolerant cultivars to be grown commercially were imidazolinone-tolerant maize, selected via tissue culture and pollen mutagenesis.<sup>17</sup> The rationale for this development was that, while imidazolinones provided selective weed control in soybeans, maize was susceptible. Imidazolinone soil residues can be phytotoxic to maize; thus introducing an imidazolinone-tolerant maize cultivar

permitted farmers to perpetuate the customary maize-soybean crop rotation. Furthermore, troublesome graminicide-resistant weeds like johnsongrass (*Sorghum halepense* (L) Pers.), wild oat (*Avena fatua* F.), *Lolium multiflorum* Lam. and *S. viridis* can be selectively controlled using imidazolinone herbicides such as imazamethabenz or imazamox. Other notable examples which are currently increasing the area sown each year to herbicide-tolerant crops include glyphosate-tolerant soybeans<sup>18</sup> and bromoxynil-tolerant cotton.<sup>19</sup>

In Canada, herbicide-tolerant rapeseed cultivars have become available in a widespread manner. The target herbicides are glyphosate, glufosinate and imazethapyr. For the non-selective herbicides, glyphosate and glufosinate herbicide tolerance has been conferred via genetic transformation (transgenes) but the imidazolinone-tolerant cultivars are non-transgenic. The mechanism which has been used to introduce the herbicide-tolerance trait can influence the ability of the producer to export products derived from the herbicide-tolerant crop. Seed availability limited the sowing of glyphosate- and glufosinate-tolerant cultivars to 16 300 ha for each in 1995. By contrast, imazethapyr-tolerant rapeseed was available for 204 000 ha.

Initially, the choice of cultivars was limited to three in 1995,<sup>20</sup> but this improved for all herbicide choices such that seven were available in 1996, with many new cultivars including potentially higher-yielding rapeseed hybrids (glufosinate-tolerant) in the pipeline. The original imazethapyr-tolerant cultivar (45A71) has been reported to produce slightly lower oil and protein contents than other rapeseed cultivars, but a new cultivar (46A72) promises to improve this characteristic.<sup>21</sup>

#### 2.1.3 Implications for crop management

A few research projects have recently considered the integration of existing (selective) herbicide treatments with the new opportunities available in herbicide-tolerant cultivars of corn and soya with non-selective herbicides. In this context Doll<sup>22</sup> determined in field trials that control of the serious weed hemp dogbane (*Apocynum cannabinum* L.) was most effective when 2,4-D and rimsulfuron were applied late post-emergence in no-till corn. As an alternative strategy, similar levels of hemp dogbane control (>90%) were also achieved in glyphosate-tolerant soybeans where glyphosate was applied in the early and full-flower stages of hemp dogbane. Sequential treatments of glyphosate gave excellent dogbane control with little reinfestation the following year. In the control of an annual weed spectrum in no-till soybeans, Harvey and Fischer<sup>23</sup> compared a series of pre-plant and post-planting herbicide treatments in glyphosate-tolerant soybeans. Sequential combinations of residual herbicides applied with treatments (2,4-D or glyphosate) prior to planting and post-emergence of glyphosate resulted in more consistent weed control with lower glyphosate rates over a wider

time span of applications. The costs of herbicide application were minimised, since the number of herbicide applications was not increased. Post-emergence combinations of glyphosate with residual herbicides did not significantly improve weed control compared to glyphosate applied alone. In the control of giant foxtail (*Setaria faberi* Herrm.), wild proso millet *Panicum milaceum* L.) and woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth.) in glufosinate-tolerant maize, it was necessary to use two sequential applications of glufosinate to achieve similar levels of weed control to those achieved where the pre-emergence herbicide metolachlor was followed by one application of glufosinate.<sup>24</sup>

In herbicide-tolerant rapeseed, the Canadian farmer has new options for weed control. Reliance on soil-applied residual herbicides such as trifluralin for broad-leaved weed control is reduced. Similarly reduced is a dependency upon selective post-emergence graminicides for volunteer cereal and grass weed control, especially where glyphosate or glufosinate is used. Imazethapyr, with its soil-applied residual and translocated action, is particularly effective upon problematic broad-leaved species, e.g. *Galium aparine* L. (cleavers) and *Galeopsis tetrahit* L. (hemp nettle). An additional benefit with the use of glyphosate or glufosinate is the ability to control grass weeds, e.g. *A. fatua* (wild oat), which have developed resistance to the aryloxyphenoxypropionate and cyclohexanedione graminicides. Thus it is likely that the introduction of these herbicide-tolerant cultivars will increase the opportunity to use a diverse range of herbicides in cropping practices, rather than the opposite reductionist approach much feared by opponents of these crop cultivars. In addition, it is possible that the relative ease with which emergent weeds can be controlled by non-selective herbicides may renew interest in dormant (autumn-seeded) and early (spring-seeded) herbicide-tolerant rapeseed. (Bahry, R., 1997, pers. comm.). Thus, under these prairie conditions, zero and minimum tillage systems can be further exploited to minimise soil erosion and soil moisture losses thereby maximising yield.

#### 2.1.4 Environmental impact

Rapeseed can become a serious weed problem as a volunteer, and this 'reservoir' of herbicide tolerance genes has also given rise to much concern about the adoption of herbicide-tolerant crops. In Canada, at the field level, farmers seldom have problems with volunteers from *B. napus*. Cereal crops following rapeseed are typically sprayed with phenoxy or sulfonylurea-based herbicides, and all rapeseed cultivars including the herbicide-tolerant cultivars are susceptible. In zero-till or direct-seeded crops, volunteer glyphosate-tolerant rapeseed plants could become a problem when spring, pre-planting applications of glyphosate are given to stubble fields. This problem might be resolved by mixing 2,4-D or a sulfonylurea herbicide with the

glyphosate where the rapeseed was tolerant to glufosinate or glyphosate. However, a sulfonylurea could not be usefully added to the mixture where the rapeseed cultivar was imidazolinone-tolerant.

The movement of genes in pollen from herbicide-tolerant to non-transgenic cultivars has been much studied, given the knowledge that *B. napus* might outcross with other crop and weed brassicas. Downey *et al.*<sup>25</sup> concluded that gene transfer from the three major Canadian oilseed crops *B. napus*, *B. campestris* and *B. juncea* to the weed *S. arvensis* was not achieved even under the most favourable conditions. Furthermore, no hybrids were identified from natural crossings of these species when they were co-cultivated in field conditions over a three-year period. Agriculture and Agri-Food Canada<sup>26</sup> concluded that gene flow from herbicide-tolerant rapeseed (*B. napus*) to relatives is possible and most likely with *B. campestris* (syn. *B. rapa*) but would not result in increased weediness or invasiveness of these relatives.

Subsequent research has confirmed this possibility, since glufosinate resistance genes from *B. napus* can be transferred to *B. campestris* as early as the first generation, by interspecific back-crossing under field conditions.<sup>27</sup> These authors consider such gene introgression should be taken into account when considering the transfer of new traits to rapeseed. In addition, Agriculture and Agri-Food Canada draw attention to the fact that, given the release of several transgenes for herbicide resistance, novel combinations of resistances might be expressed in the volunteer rapeseed populations.<sup>26</sup> Currently, this fear is not considered to be a concern which Canadian farmers generally recognise (Friesen, L., 1996, pers. comm.).

#### 2.1.5 Economic and marketing considerations

For North American farmers, the present choice of herbicide-tolerant crops is limited to maize, soybeans, cotton and rapeseed. It is evident that, while there is ready adoption of these new cultivar/herbicide combinations, they are at present confined to a niche market. This market is driven predominantly by providing farmers with the opportunity to grow an economically viable crop under particularly difficult weed-control conditions. Herbicide-tolerant crops can make the difference between growing and abandoning a crop in a given location. But there are associated extra costs. For example, in Canada, the typical cost of crop seed (non-herbicide-tolerant cultivar) plus a weed-control programme would be around Can \$61 ha<sup>-1</sup> in 1995.<sup>20</sup> With the herbicide-tolerant seed/herbicide combinations, the present cost is around \$86 ha<sup>-1</sup>, a premium of some 40%. The premium is normally composed of a combination of the following: the herbicide cost, the seed, seed royalties or a technology fee. In addition, it may be necessary to grow the crop under contract to the seed/herbicide supplier. This implies

stipulating the disposition of the crop and may include on-farm inspection rights for the seed supplier.

The production of crops grown from herbicide-tolerant cultivars of maize, soybeans and rapeseed in 1996 is less than 2% of total annual production.<sup>28</sup> The international export of these crops is problematic. The European Union (EU) and Japan accept transgenic soybeans for processing, but, in 1996, similar approval was not granted for genetically altered maize into the EU.<sup>28</sup> Similarly, at this time, rapeseed oil and meal derived from transgenic, herbicide-tolerant crops can be exported from Canada to Japan or the USA, but not into the EU. By contrast, commodities produced from herbicide-resistant cultivars which have been bred through non-transgenic methods, e.g. via pollen mutagenesis or in-vitro selection are not subject to segregation or export restrictions. Thus rapeseed and rapeseed oil produced from imidazolinone-tolerant cultivars can be freely exported from Canada to countries outside North America.

This current international situation regarding the lack of free trade in commodities which involve transgenic crops is critically important to the future of herbicide-tolerant crops. Firstly, because the segregation of commodities is neither desirable nor practical, given the volumes of crops and their products. Secondly, controversy in Europe regarding the desirability of accepting any food products which have even tenuous links with transgenes threatens international trade agreements. It is possible that, irrespective of the value of herbicide-tolerant crops to North American farmers, the prejudice of European consumer resistance to transgenic crops will curtail their import into Europe and hinder the availability of herbicide-tolerant cultivars to European farmers. A good example of this was the refusal in 1996 by the European Commission Regulatory Committee to allow the commercial release of a genetically engineered maize cultivar in the EU. The maize cultivar incorporates three transgenes, one for glufosinate tolerance, one for insect resistance *via* the *Bacillus thuringiensis* (*Bt*) toxin and an antibiotic (ampicillin) resistance gene linked to a bacterial promoter.<sup>29</sup> Antibiotic resistance genes are introduced as a laboratory technique to help identify and select genetically transformed cells in the new cultivar. The *Bt* gene is designed to minimise the need to apply chemical insecticides in the crop. On this occasion it was not the herbicide-tolerance gene or the *Bt* gene which were controversial. Instead, opinions were divided among the Committee about the risk of transfer of the ampicillin resistance gene from maize to gut bacteria in animals and to man. By contrast, original consideration of the release of this transgenic cultivar in the USA by the Department of Agriculture concluded that there were no new risks or any scientific basis to believe that the product was going to increase exposure of individuals or animal systems to ampicillin resistance.<sup>29</sup> In 1997,

the European Commission approved introduction of the genetically modified maize into Europe. It will be available for sale in France. From this example it is clear that to avoid this area of controversy, future transgenic crops should not retain an antibiotic resistance gene.

## 2.2 Europe

### 2.2.1 Background

There is at present little similarity in the speeds at which herbicide-tolerant crops come to the marketplace in North America and the EU. This reflects a fairly unified legislative approach to the trialling, approval and release of transgenic plants in Canada and the USA. By comparison, the legislative revisions in relation to 'the use of genetically modified organisms' (Directive: 90/219/EEC) and confirmation of 'the deliberate release of genetically modified organisms into the environment' (Directive: 90/220/EEC) have been under EU review for a prolonged period.<sup>30,31</sup> Such delays in legislation are a considerable source of frustration to the European biotechnology industry, who compare 12 biotechnology products released in the US since 1990 with only three products (two vaccines and a tobacco) approved in the EU.<sup>30</sup> Thus while European companies and farmers may anticipate the commercial release of herbicide-tolerant rapeseed, maize, sugar beet and eventually wheat, it is unlikely that commercial crops will be grown much before the year 2000.

### 2.2.2 Implications for crop management

In Europe, farmers are probably favourably disposed to the opportunity to purchase herbicide-tolerant crops. In maize, less reliance on soil-applied residual herbicides and the opportunity to add herbicide diversity to the cropping programme will be welcome. For rapeseed or wheat, given the existing wide range of herbicides available, it is hard to visualise farmers adopting herbicide-tolerant cultivars except where a special weed-control problem is evident, e.g. a perennial weed infestation. Of special value will be the opportunity to control herbicide-resistant weed biotypes, e.g. graminicide-resistant *Alopecurus myosuroides* Huds. (blackgrass), using non-selective herbicides such as glyphosate or glufosinate. Overall, careful consideration will require to be given to the integration of herbicide-tolerant crops with traditional crop-production systems in order that herbicide diversity of use is maintained to minimise selection pressures on weed population structure.

At present, it is impossible to predict the economic effects of herbicide-tolerant cultivars on cropping systems. Since trialling of herbicide-tolerant crops in Europe has been limited to small-plot situations, there are no published records where crop rotation systems have been evaluated to determine the agronomic and economic impact of herbicide-tolerant crops. Given the

complexity of herbicide and seed pricing structures in different countries, it is not possible to extrapolate directly from the embryonic North American market into Europe. Such an evaluation exercise cannot simply rely on isolated evidence submitted from each herbicide/cultivar provider. Instead, farmers will have to come to terms with choosing one or more herbicide/cultivar combination(s) and managing that input, not just within the current cropping year, but beyond into volunteer crop control, herbicide rotations and crop choice. Furthermore, many more transgenic crops are being developed to provide new characteristics in addition to herbicide tolerance, such as novel seed fatty acid compositions, F<sub>1</sub> hybrids, stress tolerance and altered maturation behaviour.<sup>31</sup> Thus, it is likely that the next decade will see the introduction of a significant number of new cropping opportunities as well as herbicide tolerance.

Considerable cooperation will be required between private and public sector research and development specialists to ensure that the advisory systems can adapt to provide the necessary information to farmers. Undoubtedly, while the opportunity to use herbicide-tolerant crops may appear to simplify aspects of weed control, this will require a greater administrative effort on the part of farmers. For example, careful record keeping will be essential where herbicide-tolerant and non-tolerant cultivars of the same crop are grown side by side. Guidelines for the labelling of herbicide-tolerant cultivars from the vendor through to the farmer (including field cropping records) and then to the commodity produced have been drafted in the UK by the National Farmers Union in consultation with the British Society for Plant Breeding. (Turner, R. G., 1996, pers. comm.).

### 3 ENVIRONMENTAL ISSUES

#### 3.1 Volunteer crops

The major concerns in Europe are in connection with volunteer crops and transgene introgression producing new multiple forms of herbicide resistance. Spread of herbicide-tolerance genes could be brought about by seed, vegetative growth or by the transfer of pollen. Volunteer crops are already considered to cause significant problems in weed control. Careful consideration must be given to the herbicide/crop combinations sold in a particular country. For example, volunteer potatoes are particularly troublesome in the UK, for which glyphosate is a valuable herbicide. However, if glyphosate-tolerant potatoes were introduced to the UK, their volunteers would undoubtedly become serious weed problems, given no satisfactory alternative herbicides for their control. By contrast, a prime candidate to establish feral populations is rapeseed. Studies

by Crawley *et al.*<sup>32</sup> concluded that glufosinate-tolerant rapeseed was no more invasive of, or persistent in, disturbed habitats than conventional cultivars. Since a glufosinate- or glyphosate-tolerant rapeseed is relatively easy to control by existing alternative herbicides (e.g. 2,4-D or sulfonylurea), release of such herbicide-tolerant crops would not compromise volunteer control.

#### 3.2 Gene introgression

##### 3.2.1 Hybridisation

It is unlikely that a crop will be introduced specifically containing two different herbicide-tolerance transgenes. However, inter- and intra-specific hybridisation can occur between crops and weedy plants, thus allowing the opportunity for novel transgenes for herbicide resistance to become expressed in agricultural and non-agricultural ecosystems. In the UK, the probability of gene flow from a crop to a wild species has been assessed by Raybould and Gray<sup>33</sup> in three categories. The minimal opportunity category includes potato, wheat, maize, tomato, cucumber and grain legumes. The second category (low probability) includes rapeseed, flax, raspberry and barley. Of special concern is rapeseed, since herbicide-tolerant cultivars of this crop are likely to be the first grown on a widespread scale in the UK. It has already been acknowledged in Canada that potential introgression of herbicide tolerance is most likely to occur with *B. rapa* = *B. campestris*.<sup>26</sup> However, the production of such relatives was not considered to result in increased weediness or invasiveness. In Denmark, Jorgensen and Anderson<sup>34</sup> and Mikkelsen, Andersen and Jorgensen<sup>27</sup> have shown that *B. campestris*-like plants with 20 chromosomes, a high pollen fertility, and carrying a transgene from rapeseed, can be produced as early as in the first-backcross generation, by interspecific backcrossing under field conditions. The occurrence of fertile, transgenic weed-like plants after just two generations of hybridization and backcrossing suggests a possible rapid spread of genes from rapeseed to the weedy relative *B. campestris*, and this should be taken into account when considering the consequences of transferring new traits to rapeseed.

In the third category, there is a high probability of gene flow in crops including carrots, sugar beet, cabbage, trees and forage grasses. It is worth remembering that although successful pollination may take place, hybrid plants may not be established.<sup>35</sup>

Genes which modify reproductive behaviour could alter the category within which each crop plant is placed and thereby the environmental impact.<sup>36</sup> In addition, the probability of gene flow from crops to weedy relatives will be under a strong geographical influence. Therefore, no real generalisations can be made regarding the placement of crops in the UK groupings (after Raybould and Gray<sup>33</sup>) since each

country will require to predict the associations in advance.

### 3.2.2 Ecological impact

Providing sound practical advice on the movement and impact of herbicide-tolerance transgenes based on our existing experience is rather difficult. Certainly, knowledge of the reproductive behaviour of crops from experimental evidence can be useful.<sup>37</sup> For example, some information from classical isolation studies and seed multiplication protocols might be appropriate.<sup>38</sup> More recently, Rogers and Parkes<sup>31</sup> have reviewed the likelihood of transgenes from genetically modified crops transferring to natural plant populations or non-transgenic agricultural crops. Overall, it is at present difficult to forecast transgene movement, given the variability and difficulty in interpreting the currently limited research. One particular difficulty concerns the scaling of experiments. Typically, many studies have been undertaken in small plots and may not readily be extrapolated to the field scale.<sup>39</sup> As a consequence of transgene movement into the environment, the main concerns in relation to herbicide-tolerance genes are whether or not the genes will influence the ecological behaviour of the crop, its volunteers or wild relatives. Limited research has begun to examine this topic in relation to invasiveness potential. Crawley *et al.*<sup>32</sup> compared the demography of glufosinate-tolerant rapeseed with non-modified rapeseed to assess the effect of the transgene. Field experiments examined seed dormancy, germination, plant survival and reproductive output over a three-year period. Under no environmental or experimental conditions did the transgenic cultivars exhibit different rates of population growth from those of their non-modified counterparts. Clearly, this research produced results appropriate only to a specified herbicide-tolerance transgene and a predominantly self-pollinating crop. The escape of transgenes through pollen or hybridisation was not investigated.

Recently, Ahl Goy and Duesing<sup>40</sup> used an interpretation of 1993 European field trials data to assess the impact of gene transfer to wild relatives. These authors defined environmental impact of gene transfer from genetically modified plants to wild relatives through pollen as 'probability of transfer  $\times$  consequence of transfer = potential environmental impact'. The probability of gene transfer from a crop to a wild relative was assessed such that 91% of the trials were likely to have minimal, if any, potential impact on the environment. The remaining 9% were assigned to the low-risk category and are subject to review on a case-by-case basis. The authors suggested that, if required, field evaluations of the genetically modified plants could be performed to ensure reproductive isolation by preventing flowering, by limiting pollen dissemination, and/or controlling wild relatives within the area and local vicinity of the plot. Similarly, Ellestrand and Hoffman<sup>41</sup>

have proposed isolation distances and cultural methods which reduce the opportunity for flowering periods of potentially sexually compatible plants to coincide. While these suggestions may have theoretical credibility, in a practical cropping environment they are probably impractical and would certainly not encourage farmers to adopt herbicide-tolerant crops. In practice, the containment of transgenes is probably quite impractical, therefore, alternative strategies require to be considered.

It is worth considering using the instability of transgenes as a mechanism whereby the functional lifespan of a novel transgene such as herbicide tolerance will be finite. It is already known that new phenotypes created by the introduction of foreign DNA into plants can be unstable following propagation, leading to a loss of the newly acquired trait.<sup>42</sup> In nearly all cases where this phenomenon has been investigated at the molecular level, loss of expression does not correlate with loss of the transgene, but rather with its inactivation.<sup>43,44</sup> A range of opportunities which might reduce the infertility of crop plants with wild relatives is reviewed by Keeler *et al.*,<sup>45</sup> including autocidal traits expressed in crops and triggered by exposure to low temperatures. Gressel<sup>4</sup> suggested that genetic engineers are attempting to design self-destruct features into gene constructs so that tolerance genes will be inactivated or be excised from the genotype after one or two generations. He concludes that this type of fail-safe device would prevent a volunteer weed problem and protect the seed companies from farm-saved seed. There is no doubt that, given the commercial investment in transgenic crops, including herbicide tolerance genes, biotechnology companies are likely to seek sophisticated ways to protect their intellectual property. Thus the introduction of a mechanism which would at least silence a transgene in a non-hybrid crop may soon become a commercial necessity. So far this opportunity to minimise the environmental impact of transgene 'escape' in the environment while retaining the commercial advantages of the herbicide-tolerant crop by designing self-destructing transgenes has not been reported.

### 3.2.3 Transgene detection

If we assume that the first generation of transgenes for herbicide tolerance will be stable, how will these genes be monitored in the environment? Depending on how the regulations develop in the EU there may be no statutory provision. Once a cultivar is sold commercially there could be no follow-up. Certainly, a range of sophisticated molecular methods exists for the detection of transgenes including herbicide-tolerance transgenes (reviewed by Rogers and Parkes<sup>31</sup>). These diagnostic techniques normally use polymerase chain reaction (PCR) to probe known DNA sequences.<sup>46</sup> A PCR assay has been used to monitor transgenes from trials with rapeseed and sugar beet.<sup>47</sup> These authors

found that the assay could detect the transgenes in different plant tissues (up to 1000 plants could be pooled, thus reducing the number of DNA extractions required) and pesticide residues did not inhibit the PCR assay. While these molecular techniques can be simplified and automated to the extent that multiple samples can be assessed, they are unlikely to be used as a routine diagnostic tool.<sup>48</sup> Alternatively, plants or seed can be screened by applying the test herbicide or herbicides.<sup>49</sup> This can be slow and haphazard where a screening programme for multiple herbicide tolerances is required, but this might be the only thorough diagnostic test where multiple herbicide tolerances develop via introgression/hybridisation.

An alternative strategy to consider is the incorporation of a new gene coding for green fluorescent protein (GFP) as a neutral marker gene linked to the herbicide-tolerance transgene.<sup>50</sup> In preliminary studies, the leaves of transgenic plants engineered with the GFP gene fluoresce green when exposed to ultraviolet or blue light under low ambient light. The greenish fluorescence is a contrast to the pinkish hue emitted by non-transgenic plants. Thus the GFP gene is unique as a reporter gene, since it has an ability to fluoresce in the absence of added substrate, enzyme or cofactor.<sup>51</sup> In ecological studies where the detection of a single herbicide-tolerance transgene was being assessed in crop and weedy relatives, the GFP could be especially useful in providing a rapid and inexpensive diagnostic method. Incorporated in association with a wide range of transgenes in various crops, its specific utility would be lost, assuming multiple transgenic crops were grown in one cropping environment. It would then become a general marker for transgenic plants, a preliminary screening mechanism which could be useful in identifying plant genomes for further genetic analysis.

#### 4 CONCLUSIONS

Over the last decade, advances in biochemistry, physiology and molecular biology have enabled the selection, production and commercialisation of herbicide-tolerant crops. Indeed, it is easy to become so entranced with the effectiveness of this elegant technology that we lose sight of its rationale in crop production. Undoubtedly, given the huge costs associated with the discovery of new herbicides, the engineering of resistance to existing herbicides in crops may represent an attractive option to industry. But perhaps this is still a short-term solution to a long-term difficulty. Certainly, it is prudent to question the perceived benefits of this technology to its users and the consumer.

This question has become more controversial as herbicide-tolerant crops come close to the market place and political influences are brought to bear. Recent evidence would suggest that EU policies are moving to

become more aligned with those of our more liberalised American counterparts. Thus environmentalists fear that commercial pressures upon government to create a competitive international business environment will hasten the field release of a greater number of herbicide-tolerant crops without adequate safeguards. These releases would progress directly from the scale of small plots and annual assessment to full commercial field release. Farmers will require, via independent assessments, to be convinced of the need and technical and economic benefits associated with the adoption of herbicide-tolerant crops. In addition, considerable extension advice will be required to ensure that herbicide-tolerant crops, transgenics and conventional crops can be grown and managed by farmers with due regard for environmental issues and sustainable cropping objectives.

Against this backdrop of inevitable commercial release it is likely that voluntary programmes of environmental audit will be undertaken. These audits might usefully record the impact of the introduction of herbicide-tolerant crops upon herbicide use patterns, the evolution of weed floras and the nature of volunteer crop problems. In addition, the movement of herbicide tolerance genes in the cropping and non-cropping environments could be monitored, with special attention to associated crop species and wild relatives. Supplementing existing seed/seedling screening with herbicides and molecular-based tests with novel rapid diagnostic assays for herbicide-resistance transgenes would also be beneficial in building up a reliable data base. Such a body of scientific information collected for a specific region or country would be of special value as a blueprint for transgene behaviour. Consequently, this information could be used when different types of transgenic crops are being considered for more general release.

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